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Hypocholesterolemia in Hairy Cell Leukemia: A Marker for Proliferative Activity

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Hypocholesterolemia is a well-documented phenomenon associated with a variety of hematological malignancies and nonmalignant disorders associated with splenomegaly. To determine the incidence of hypocholesterolemia in patients with hairy cell leukemia (HCL), we measured the serum cholesterol levels before and after a single cycle of 2-chlorodeoxyadenosine (2-CdA) in 46 patients. The mean pre-treatment serum cholesterol level was 152.8 mg/dl (range, 60 to 293 mg/dl). The mean post-treatment serum cholesterol level was 190.0 mg/dl. This was significantly higher than the pre-treatment values ($P < 0.0001$). Twelve patients who had previously undergone splenectomy showed a similar response to treatment, with a pre-treatment value of 180.0 mg/dl and a post-treatment value of 219.8 mg/dl ($P < 0.0001$). However, there was a significant difference in the pre-treatment serum cholesterol levels in the nonsplenectomized patients (143.0 mg/dl) compared to the splenectomized patients (180.0 mg/dl) ($P < 0.03$). The pre-treatment serum cholesterol did not correlate with the pre-treatment splenic index (correlation coefficient = −0.39, $P < 0.065$). Similarly, there was no correlation between the change in splenic index and the change in serum cholesterol level post-treatment. These findings suggest that hypocholesterolemia in HCL is related to tumor burden and not to splenomegaly alone. Since cholesterol is critical to hairy cell metabolism and structure, treatment strategies interfering with cholesterol synthesis may be productive. Am. J. Hematol. 55:129–133 1997.

Key words: hypocholesterolemia; hairy cell leukemia; proliferative activity

INTRODUCTION

Hypocholesterolemia has been observed in patients with a variety of hematologic malignancies, including myeloproliferative disorders [1–4], acute myeloid leukemia [5–7], acute lymphocytic leukemia [8,9], chronic lymphocytic leukemia (CLL) [10], and nonmalignant disorders associated with splenomegaly [11]. The cause of hypocholesterolemia in these diseases has not been determined. In myeloproliferative disorders, studies have suggested that splenomegaly leads to hypocholesterolemia through a ''scavenger pathway'' whereby activated macrophages increase receptor-mediated low-density lipoprotein (LDL) degradation or increased LDL catabolism [1,3,12].

Patients with hairy cell leukemia (HCL) often have marked splenomegaly [13]. Therefore, this study was designed to evaluate the incidence of hypocholesterolemia in patients with HCL and to determine if serum cholesterol levels change following effective treatment with 2-chlorodeoxyadenosine (2-CdA). If so, the serum cholesterol level may be a marker for proliferative activity.

MATERIALS AND METHODS

Patient Population

Between February 1991 and December 1994, 50 patients with HCL were treated at the Robert H. Lurie Cancer Center of Northwestern University and form the basis for this report (Table I). Eligibility criteria included the following: (1) pathologically confirmed diagnosis of HCL based on the bone marrow aspirate, core biopsy, and peripheral blood smear; (2) evidence of active dis-

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TABLE I. Patient Characteristics

Characteristics	No. of patients
	46
Mean age in years (range)	56 (40–85)
Sex	
Male	39
Female	7
Previous treatment	
None	27
Splenectomy alone	6
Interferon	7
Splenectomy, interferon	4
Splenectomy, interferon, 2-deoxycoformycin	2

ease, including any of the following: neutropenia (neutrophils <1,500/μl), anemia (hemoglobin <12 g/dl), thrombocytopenia (platelets <150,000/μl), symptomatic splenomegaly or adenopathy; (3) no active infection; (4) no prior treatment for the disease within 4 weeks of study entry; (5) normal hepatic and renal function.

Forty-six of the 50 patients had both pre-treatment and post-treatment serum cholesterol levels measured. There were 39 males and 7 females. The ages ranged from 40 to 85 years (median, 60). Twenty-seven patients were previously untreated, and nineteen patients were previously treated. Twelve patients were previously treated with splenectomy, six with splenectomy alone, four with splenectomy and then interferon, and two with prior splenectomy, interferon then 2′-deoxycoformycin. Seven patients had received only prior interferon.

Patients were treated with 2-CdA provided by the R.W. Johnson Pharmaceutical Research Institute (Raritan, NJ). All patients received 2-CdA at a dose of 0.1 mg/kg/d by continuous intravenous infusion for 7 days. No patients received hematopoietic growth factors. Patients received a single 7-day cycle of 2-CdA and were then evaluated at 3 months. If they achieved a partial remission (PR), they were eligible to receive a second 7-day cycle. Detailed characteristics of the first 20 patients have been previously reported [14].

Serum Cholesterol Measurement

All patients enrolled in this study had a random serum cholesterol level included in their screening laboratory studies within 1 week prior to treatment. As this was a retrospective study, serum LDLs, high-density lipoproteins, and triglycerides were not routinely obtained and thus not included in the data. Cholesterol levels were obtained 3 months after treatment from 46 of the 50 patients. All patients were well nourished, had normal serum albumins, and were without liver disease, diabetes, or clinical signs or symptoms of thyroid disease. There were no dietary restrictions during the treatment.

Splenic Index Measurement

Abdominal computed tomography scans were obtained on all patients prior to and 3 months after treatment. Splenomegaly was assessed by measuring the splenic index. which is reported to be a more accurate measurement of spleen size [15]. The splenic index was determined by calculating the product of the craniocaudal dimension, the anterior-posterior thickness, and the transverse thickness.

Hairy Cell Index Measurement

The hairy cell index is a measurement of the amount of infiltration of the bone marrow by hairy cells. It is defined as a product of the cellularity of the bone core biopsy and the fraction of hairy cells present in the bone core biopsy. It is expressed in a number from 0 to 1 [16].

Statistical Analysis

Change in serum cholesterol, hairy cell index, and splenic index were analyzed using the paired *t*-test. Cholesterol levels were compared between splenectomized and nonsplenectomized patients using the unpaired *t*-test. The Pearson correlation coefficient was used to correlate cholesterol levels with splenic index and hairy cell index. Significant correlation was tested using the *t*-test for zero correlation. In all analyses, significance was defined as $P = 0.05$ [17].

RESULTS

Serum Cholesterol Levels Before Treatment With 2-CdA (Fig. 1)

The mean pre-treatment serum cholesterol level of all patients was 152.8 mg/dl, with a median of 155 mg/dl (range, 60 to 293 mg/dl). This value is considerably lower than the mean cholesterol of the healthy American population (210 mg/dl) [18]. The mean serum cholesterol level of patients previously treated with splenectomy was 180.0 mg/dl, with a median of 176.5 mg/dl (range, 95 to 293 mg/dl). The mean serum cholesterol level of nonsplenectomized patients was 143.1 mg/dl, with a median of 143 mg/dl (range, 60 to 203 mg/dl). The mean serum cholesterol of nonsplenectomized patients was significantly lower than those who had undergone splenectomy ($P = 0.03$).

Serum Cholesterol Levels After Treatment With 2-CdA

The mean post-treatment serum cholesterol level of all patients increased significantly from a mean of 152.8 mg/dl (median, 155 mg/dl) to 193.0 mg/dl (median, 188.5 mg/dl) ($P < 0.0001$). An increase in serum cholesterol levels was noted in both splenectomized and nonsplenectomized patients. Nonsplenectomized patients had an increase in serum cholesterol levels from 143.1 mg/dl (median, 143 mg/dl) to 183.6 mg/dl (median, 180 mg/dl) ($P < 0.0001$). Those treated with splenectomy showed an increase in serum cholesterol from a mean of

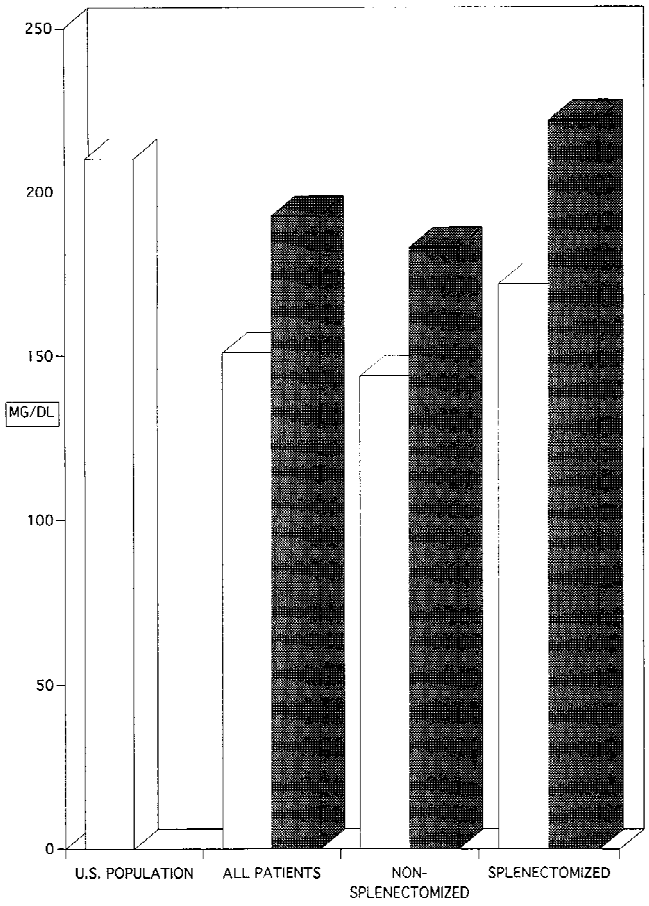

Fig. 1. Change in mean serum cholesterol following one cycle of 2-CdA. Unshaded = pre-treatment; shaded = post-treatment.

180.0 mg/dl (median, 176.5 mg/dl) to 219.8 mg/dl (median, 225.5 mg/dl) ($P < 0.0001$). The increased cholesterol level was sustained after discontinuation of the drug.

Relationship Between Serum Cholesterol and Splenic Index

The splenic index was determined in 23 of 38 non-splenectomized patients. The splenic index decreased from an initial pre-treatment mean of 2,381 cubic cm (range, 660 to 7,128 cubic cm) to a mean of 994 cubic cm (range, 239 to 1,728 cubic cm) post-treatment ($P < 0.0001$) (Fig. 2). The pre-treatment serum cholesterol was not correlated with the pre-treatment splenic index (Pearson correlation coefficient = −0.39, $P = 0.065$) (Fig. 3). There was also no correlation between the change in splenic size/index and change in serum cholesterol (parametric correlation coefficient = 0.0806, $P = 0.72$).

Relationship Between Serum Cholesterol and Hairy Cell Index

The hairy cell index was determined in 46 of 50 patients in the study. The mean hairy cell index before

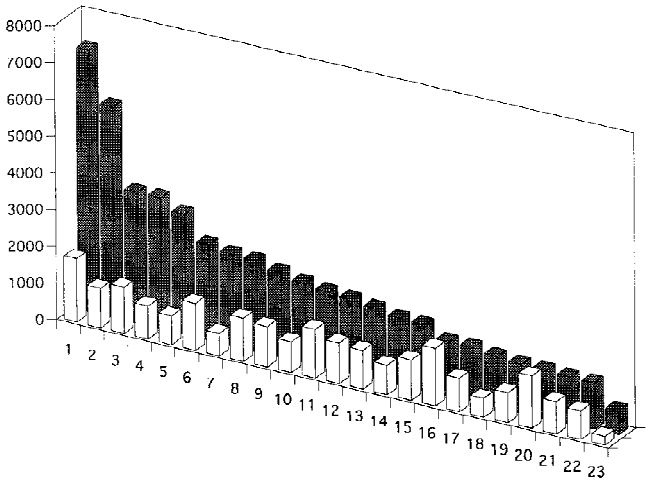

Fig. 2. Change in splenic index following one cycle of 2-CdA. Y axis = splenic index in cm cubed; X axis = patients. Shaded area = pre-treatment splenic index; unshaded = post-treatment splenic index.

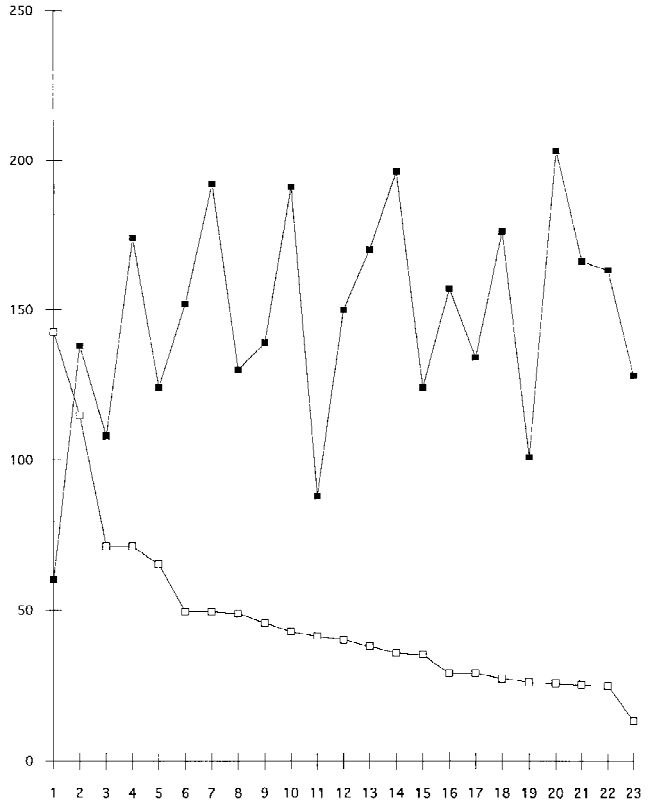

Fig. 3. Correlation between initial splenic index and mean serum cholesterol. The correlation coefficient is = −0.39 (p = 0.064). Shaded box-line = initial serum cholesterol in mg/dl. Unshaded box-line = initial splenic index in cm cubed. Splenic index is scaled down to SI/50.

treatment with 2-CdA was 0.455. Post-treatment hairy cell index was 0.0, as all patients were disease-free post-treatment. The pre-treatment hairy cell index was not correlated with the initial serum cholesterol (Pearson correlation coefficient = −0.192, $P = 0.202$). There was also no correlation between the change in serum cholesterol and hairy cell index (Pearson correlation coefficient = 0.186, $P = 0.226$). However, there was a trend noted: as hairy cell index decreased to 0 post-treatment, the serum cholesterol increased post-treatment.

DISCUSSION

The pathogenesis of hypocholesterolemia in patients with HCL is not completely understood. Hypocholesterolemia in our patient population is not explained by malnutrition. All patients in this study had normal serum albumin levels, had not lost weight, and appeared well before therapy. Cholesterol levels were not obtained in the fasting state; however, the mean values of nonfasting total cholesterol levels exhibit negligible differences from the mean of fasting levels [19]. Serum cholesterol levels are commonly low in other leukemias. Despite an increased rate of cholesterol biosynthesis within the leukemic cells [20,21], the cholesterol content of these cells is low. This paradox is attributable to the need of these rapidly dividing leukemic cells to endow their progeny with cholesterol-containing membrane constituents. In contrast, hairy cells have been shown to have an increased cholesterol content [22,23]. Yachnin and colleagues showed that hairy cells synthesize cholesterol at a rate five to six times higher than peripheral blood mononuclear cells (PBMNC) [24]. An elaborate set of experiments suggests that the increased cholesterol content in hairy cells is not due to an increased proliferative rate, nor to increased loss of newly synthesized cholesterol into the serum. It is suggested that the increased cholesterol content is required to support the redundant plasma membrane that is present in hairy cells.

Juliusson and colleagues also recently reported hypocholesterolemia in patients with untreated HCL [25]. The cholesterol level rose following successful treatment with 2-CdA. Low-density lipoprotein receptor activity in HCL cells was elevated in only one of 12 patients, suggesting that increased LDL-receptor activity is not responsible for lowering serum cholesterol. Their data also showed that serum cholesterol was inversely related to spleen size and therefore suggested that splenomegaly was involved in the pathogenesis.

It has been suggested that splenomegaly contributes to hypocholesterolemia in hematologic disorders. Aviram et al. studied LDL levels after splenectomy in patients with myeloproliferative disorders [3]. The results supported a relationship between splenic size and cholesterol levels, but no etiology was identified. It was postulated that a macrophage-induced scavenger pathway in the spleen increased LDL catabolism. Gilbert and Ginsberg also investigated decreased cholesterol in myeloproliferative disorders and its association with splenomegaly [4]. In chronic myelocytic leukemia, LDL levels were inversely related to leukocytosis and spleen size. Even after splenectomy, LDL levels cycled with response to chemotherapy, suggesting a relationship to disease burden rather than to a passive physiologic mechanism occurring in the spleen.

The availability of an extremely effective agent such as 2-CdA, which induces remission in the majority of patients, provided the opportunity to correlate disease activity with serum cholesterol. The data reported here suggest that hypocholesterolemia is not due to a unique pathogenic mechanism in the spleen, but rather is related to disease burden. Mean cholesterol levels increased significantly from 152.8 mg/dl pre-treatment to 193.0 g/dl post-treatment, as tumor burden decreased and remission was achieved. This significant rise was seen in patients both with and without splenectomy. Although cholesterol levels were lower in nonsplenectomized patients, splenectomized patients still had significant hypocholesterolemia. It is likely that patients with enlarged spleens had more profound hypocholesterolemia due to the increased tumor burden provided by the spleen acting as a disease reservoir. This is consistent with the data reported by Yachnin and colleagues, which showed that the rate of cholesterol biosynthesis in spleen cells from two patients was similar to that present in the same patient's peripheral hairy cells [24].

The increased cholesterol content in hairy cells may provide an understanding of the marked sensitivity of HCL to purine analogs relative to other lymphoproliferative disorders. Kawasaki et al. showed that both deoxycytidine kinase (dCK) and cytoplasmic 5′nucleotidase (5′-NT) levels were determinants of 2-CdA responsiveness [26]. In patients with CLL and HCL, 2-CdA responders have significantly higher dCK levels and lower 5′-NT levels than 2-CdA nonresponders. However, they also found that the HCL cells had mean dCK levels three to five times lower than CLL cells despite the fact that HCL cells were much more responsive than CLL cells to 2-CdA. These results indicate that other factors independent of dCK may be responsible for the marked responsiveness of HCL to 2-CdA. Lechleitner et al. recently studied the in vitro effect of 2-CdA on cellular lipid metabolism in a macrophage line [27]. 2-CdA produced a dose-dependent reduction in cholesterol content and in the extracellular incorporation of [14c] oleic acid into the cholesterol esther fraction. 2-CdA did not effect intracellular cholesterol synthesis as measured by the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity, and thus its effect is presumed to be based on interference with acetylated low-density lipoprotein me-

tabolism. Thus, the increased cytotoxicity of 2-CdA in hairy cells may be attributable to its lipid-altering effect. It is provocative that the gene encoding HMG-CoA reductase is located on chromosome 5 at 5q13-q32 [28] and clonal chromosomal abnormalities involving chromosome 5, including interstitial deletions involving 5q13, occur in 40% of patients with HCL [29].

Since cholesterol is critical to hairy cell metabolism and structure, treatment strategies interfering with cholesterol synthesis may be a new direction to pursue. Indeed, in vitro data suggest that cholesterol synthesis inhibitors such as lovastatin may be useful in patients with hematologic malignancies [30,31]. Optimal serum cholesterol levels might be determined in which hairy cell function and metabolism are inhibited without disturbing normal cell growth. Since some patients with HCL may eventually relapse after 2-CdA or other effective treatments [32], novel strategies, which might include a cholesterol synthesis inhibitor, may warrant further investigation.

REFERENCES

1. Gilbert HS, Ginsberg H, Faegerstrom R, Brown WV: Characterization of the hypocholesterolemia in myeloproliferative disease: Relation to disease manifestation and activity. Am J Med 71:595–602, 1981.
2. Ginsberg H, Gilbert HS, Gibson JC, Le NA, Brown WV: Increased low-density lipoprotein catabolism in myeloproliferative disorders. Ann Intern Med 96:311–316, 1982.
3. Aviram M, Brook JG, Tatarsky I, Levy Y, Carter A: Increased low-density lipoprotein levels after splenectomy: A role for the spleen in cholesterol metabolism in myeloproliferative disorders. Am J Med Sci 29:25–28, 1986.
4. Gilbert HS, Ginsberg H: Hypocholesterolemia as a manifestation of disease activity in chronic myelocytic leukemia. Cancer 51:1428–1433, 1983.
5. Budd D, Ginsberg H: Hypocholesterolemia and acute myelogenous leukemia. Cancer 58:1361–1365, 1986.
6. Reverter CJ, Sierra J, Msrti-Tutudsaus JM: Hypocholesterolemia in acute myelogenous leukemia. Eur J Haematol 41:317–320, 1988.
7. Porta C, Moroni M, Nastasi G: Hypocholesterolemia and acute myeloid leukemia (AML). Haematologica 76:348, 1991.
8. Shokunni WA, Ahaneku JE, Okpala IE: Cholesterol, lipoprotein and phospholipid levels in acute lymphoblastic leukemia. Eur J Haematol 46:59–61, 1991.
9. Baroni S, Scriban D, Paguno L, Zuppi C, Leone G, Giardina B: Lipids and lipoproteins in acute lymphoblastic leukemia (ALL). Leukemia Res 18:643–644, 1994.
10. Marini A, Carulli G, Azzara A, Grassi B, Ambrogi F: Serum cholesterol and triglycerides in hematologic malignancies. Acta Haematol 81:75–79, 1989.
11. Rifkind BM, Gale M: Hypolipidemia in anemia. Cancer 2:640–644, 1967.
12. Traber MG, Kayden HS: Low-density lipoprotein-receptor activity in human monocyte-derived macrophages and its relation to atheromatous lesions. Proc Natl Acad Sci 77:5466–5470, 1980.
13. Golomb HM, Catovsky D, Golde DW: Hairy cell leukemia: A clinical review based on 71 cases. Ann Intern Med 89:677–683, 1978.
14. Tallman MS, Hakimian D, Variakojis D, Koslow D, Sisney GA, Rademaker AW, Rose E, Kaul K: A single cycle of 2-chlorodeoxyadenosine results in complete remission in the majority of patients with hairy cell leukemia. Blood 80:2203–2209, 1992.
15. Cools L, Osteaux M, Divano L, Jeanmart L: Prediction of splenic volume by simple CT measurement: A statistical study. J Comput Assist Tomogr 7:426–430, 1983.
16. Golomb HM, Vardiman J: Response to splenectomy in 65 patients with HCL: Evaluation of spleen weight and bone marrow involvement. Blood 61:349–352, 1983.
17. Rosner B: ''Fundamentals of Biostatistics,'' Ed. 4. Belmont, CA: Duxbury Press, 1994.
18. Anonymous: Trends in serum cholesterol levels among US adults aged 20 to 74 years. Data from the National Health and Nutrition Examination Surveys, 1960–1980. National Center for Health Statistics-National Heart, Lung and Blood Institute Collaborative Lipid Group. JAMA 257:937–942, 1987.
19. Cooper GR, Meyer GL, Smith SJ, Schlant RC: Blood lipid measurements: Variations and practical utility. JAMA 267:1652–1660, 1992.
20. Chen HW, Kandutsch AA, Heiniger HJ: The role of cholesterol in malignancy. Prog Exp Tumor Res 22:275–316, 1978.
21. Ho YK, Smith G, Brown MS, Goldstein JL: Low density lipoprotein (LDL) receptor activity in human acute myelogenous leukemia cells. Blood 52:1099–1114, 1978.
22. Liebes LF, Pelle E, Zucker-Franklin D, Silber R: Comparison of lipid composition and 1,6-diphenyl-1,3,5,hexatriene fluorescence polarization measurements of hairy cell with monocytes and lymphocytes from normal subjects and patients with chronic lymphocytic leukemia. Cancer Res 41:4050–4056, 1981.
23. Golomb HM, Safford CW, Nathans AH, Dawson G: Phospholipid and cholesterol differences amongst leukemic cell types with special reference to hairy cell leukemia: A preliminary report. Clin Chem Acta 116:311–318, 1981.
24. Yachnin S, Larson RA, West JE: Rates of cholesterol biosynthesis are related to early differentiation in acute nonlymphocytic leukemia cells. Br J Haematol 54:459–466, 1983.
25. Juliusson G, Vitols S, Liliemark J: Mechanisms behind hypocholesterol in hairy cell leukemia. BMJ 311:27, 1995.
26. Kawasaki H, Carrera CJ, Piro LD, Saven A, Kipps TJ, Carson DA: Relationship of deoxycytidine kinase and cytoplasmic 5′-nucleotidase to the chemotherapeutic efficacy of 2-chlorodeoxyadenosine. Blood 81:597–601, 1993.
27. Lechleitner M, Auer B, Zilian U, Hoppicher F, Schirmer M, Foger B, Geisen F, Patsson JR, Konwalinka G: The immunosuppressive substance 2-chlor-2-deoxyadenosine modulates lipoprotein metabolism in a murine macrophage cell line (p388 cells). Lipids 29:627–633, 1994.
28. Mohandas T, Heinzman C, Sparkes RS, Wasmuth J, Edwards P, Lusis AJ: Assignment of human 3-hydroxy-3-methylglutaryl coenzyme A reductase gene to q13–q23 region of chromosome 5. Somatic Cell Mol Genet 12:89–94, 1986.
29. Haglund U, Julivsson G, Birgitta S, Gosta G: Hairy cell leukemia is characterized by clonal chromosome abnormalities clustered to specific regions. Blood 83:2637–2645, 1994.
30. Vitols S, Angelin B, Ericsson S, Garhton G, Juliusson G, Masquelier M, Paul C, Peterson C, Rudling M, Soderberg-Reid K, Tidefelt V: Uptake of low-density lipoprotein by human leukemic cells in vivo: Relation to plasma lipoprotein levels and possible relevance for selective chemotherapy. Proc Natl Acad Sci USA 87:2598–2602, 1990.
31. Bansal N, Houle AG, Melnykovych G: Comparison of dexamethasone and lovastatin (Mevinolin) as growth inhibitors in cultures of T-cell derived human acute leukemic cell lines (CEM). Leukemia Res 13: 875–882, 1989.
32. Seymour JF, Kurzrock R, Freireich EJ, Estey EH: 2-chlorodeoxyadenosine induces durable and prolonged suppression of CD4+ lymphocyte counts in patients with hairy cell leukemia. Blood 83:2906–2911, 1994.